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BACTERIA IN ENGINEERING.

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PROFESSOR OF PATHOLOGY AND BACTERIOLOGY.

SINCE progressive engineering has promoted a purer water supply, efficient sewerage, and cleanliness all about us, the sanitary engineer has become a recognized benefactor; sanitary engineering concerns itself much with the disintegration of organic matter, the disposal of debris, the cause and spread of disease, as likewise its prevention, so that the study of bacteria seems to adapt itself well to the training of the engineer.

In order to properly interpret the relations which exist between the study of micro-biology and that of engineering, it is necessary to consider the general characteristics of that class of lower organisms known as bacteria, and in the study of their vital processes, find the explanation for the evidences of bacterial activity as they come within the field of engineering.

Bacteria are among the smallest of all known living organisms, the largest of them having a diameter of only a few micro-millimeters, while the smallest do not measure more than a fraction of a micro-millimetre. Structurally and morphologically they are extremely simple, though biologically very variable. Through their ability to derive their carbon from tartrates and their nitrogen from ammonia or its salts, they are ranked in the vegetable kingdom. They obtain their food entirely through the surface absorption of soluble nutritious substances. They are reproduced by transverse division, and in some respects resemble the fungi, hence are called fission fungi, or schizo-mycetes. They are also closely allied to certain kinds of algæ, though they must receive their

nourishment from living or dead organic material, since they are without chlorophyll, the green coloring matter possessed by the higher plants, by means of which they are enabled, in the presence of sunlight to decompose CO_2 NH_3 and H_2S into their elementary constituents.

Bacteria, especially the motile forms are also closely allied to some of the micro-organisms which belong to the animal kingdom.

Bacteria may be defined as extremely minute vegetable organisms, consisting of single spherical, rod-shaped, or corkscrew-like cells or aggregates of such cells, between whose protoplasm and nucleus it has been as yet impossible to differentiate with certainty.

Bacteria occur as saprophytes or refuse eaters, and as parasites. Saprophytic bacteria are such as commonly exist independently of a living host, obtaining their supply of nutriment from soluble food stuffs in dead organic matter. Parasitic bacteria, on the other hand, live on or in some other organism, from which they derive their nourishment for the whole or a part of their existence.

Those bacteria which depend entirely upon a living host for their existence are known as strict parasites; those that can lead a saprophytic existence, but which can also thrive within the body of a living animal, are called facultative parasites. The strict saprophytes, which represent the large majority of all bacteria, while they destroy refuse, are not only harmless to living organisms but perform many important functions in nature without which existence would be impossible, such as the destruction of dead organic material through decomposition, putrefaction, and fermentation.

The parasites, on the other hand, though some of them may multiply in the secretions, or on the surface of the body without injury to the animal upon which they depend for their existence, are usually harmful invaders, giving rise through the lesions brought about in the body tissues by their growth and products, to derangements which are known as acute or chronic infectious diseases.

The basic forms of the single bacterial cells are three fold—the sphere, the rod, and the segment of a spiral. Although under different conditions the form of any one species may vary considerably, yet these three main divisions under similar conditions are permanent; and, so far as we know, it is never possible by any means, to bring about changes in the organisms that will result in the conversion of the morphology of the members of one group into that of another—that is, micrococci always, under suitable conditions, produce micrococci, bacilli produce bacilli, and spirilla produce spirilla.

The spherical form, or coccus, varies in size from about 0.3μ as minimum diameter to 3μ as maximum. According to the manner and direction of their division, pairs or diplococci, chains or streptococci, groups of four—tetrads, packets in cubes—sarcinæ, or irregularly shaped grape-like bunches, called staphylococci are formed; the micrococcus ureæ, diplococcus of pneumonia, and streptococcus of erysipelas are examples of this group.

The type of the rod form, or bacillus is a cylinder, the length being always longer than its breadth; the size of different varieties varies greatly from a length of 30μ and a breadth of 4μ to a length of 0.2μ and a breadth of 0.1μ ; the bacillus of typhoid, and of tuberculosis are examples of this type.

The members of the third morphologic group are spiral in shape and termed spirilla, of which the spirillum of Asiatic cholera is a representative.

Reproduction and preservation of the species occurs by cell division and spore formation.

VEGETATIVE REPRODUCTION. This takes place by the division of the original bacterial cell into two individuals; the complete process of cell reproduction in most varieties occupies under favorable conditions, about twenty to thirty minutes, so that, the increase being in a geometrical ratio, the number of individuals which might arise from a single bacterium in 3 or 4 days is almost inconceivable, and would *en masse* weigh thousands of tons; fortunately there are certain checks to such a rapid multiplication.

SPORE FORMATION. This must be distinguished from vegetative reproduction, since it is a process by which the organisms are enabled to enter a stage in which they resist deleterious influences to a much higher degree than is possible for them in a growing or vegetative condition.

The spore is a bright round or oval body which is formed within the bacterial cell, the development of which can be watched under the microscope, the bioplasm of the cell afterwards degenerates, the cell membrane ruptures, and the spore is set free. Spores are single, one only forming in each cell, and they seem to fulfill the purpose of perpetuating the race when it is threatened with extinction from adverse circumstances.

Under favorable conditions the germination of spores takes place, and the vegetative cell is again produced. A knowledge of spore formation is of practical import, since the high resisting power of the spores greatly influences the sterilization and disinfection of all matter in which bacteria are present.

CONDITIONS OF GROWTH. Although there are among the bacteria related to disease, a number which are met with only in the bodies of living animals or plants, and therefore, so far as we know, strictly parasites, yet most pathogenic or disease producing bacteria can be cultivated more or less readily in artificial culture media under suitable conditions.

The majority of bacteria which occur usually as saprophytes are easily cultivated artificially, but there are some, such as various micro-organisms found in saliva and in water, which with our present knowledge, are either difficult or impossible to cultivate.

All bacterial culture media must contain an abundance of water; salts are also indispensable, and there must be organic material as a source of carbon and nitrogen. The greater number of important bacteria and all the pathogenic species thrive best in media containing albuminoid substances and of a slight alkaline reaction.

The demands of bacteria in the composition of the culture media vary very considerably; there are some species of

water bacteria, for instance, which require so little organic material that they will grow in water that has been twice distilled.

In such cases development probably takes place owing to some contamination of the water or else through the decomposition of the ammonia and carbonic acid in the air. Few bacteria of any importance are so easily satisfied, though there are many species which are able to develop without the presence of albumen and in comparatively simple culture media, such as the culture liquid of Voges and Fraenkel, which consists of—water, 1000; sodium chloride, 5; neutral sodium phosphate, 2; ammonium acetate, 6; and asparagin, 4. In this media many bacteria grow well.

Considering the source of the more important chemical ingredients of bacteria we find that their nitrogen is most readily obtained from diffusible albuminoid material and less easily from ammonium compounds. Their carbon they derive from albumen, peptone, sugar, and other allied carbohydrates, glycerine, fat, and other organic substances.

The majority of bacteria absolutely require oxygen for their growth, but a considerable minority fail to grow unless it is excluded. A knowledge of this latter fact we owe to Pasteur, who divided bacteria into aërobic and anaërobic. Between these two groups we have those that can grow either with or without the access of oxygen.

Sulphur and phosphorus are two important food stuffs required by bacteria. Either calcium or magnesium and sodium or potassium are also usually required for bacterial growth.

Iron is demanded by but a few varieties. When we consider the more complex culture media, either those naturally existing, such as blood serum, or those created for the cultivation of bacteria, we find, beyond the necessary amount of soluble food stuffs, that the relative proportions of each form and the total concentration are of great importance.

It is nevertheless, true that very wide differences can exist with but slight effect upon the development of bacteria, the development of bacteria usually ceasing through the accumu-

lation of deleterious substances in the culture media, rather than through food exhaustion.

The reaction of the nutritive media is of very great importance. Most bacteria grow best in those that are slightly alkaline or neutral. Only a few varieties require an acid medium, and none of these belong to the parasitic bacteria. An amount of acid or alkali insufficient to prevent the development of bacteria may still suffice to rob them of some of their most important functions, such as the production of poison.

The influence of one species upon the growth of another, either when the bacteria grow together or follow one another, is very marked. The development of one variety of bacteria in a medium causes that substance, in the majority of instances, to become less suitable for the growth of other bacteria. This is due partly to the impoverishment of the foodstuff, but more to the production of chemical substances or enzymes, which are antagonistic not only to the bacteria producing them, but also to many other varieties; less frequently the changes produced by one variety of bacteria in the food stuff are favorable for some other form.

For the growth of bacteria a suitable temperature is absolutely requisite. For different varieties the most favorable temperature varies, but for all a range of about $2\frac{1}{2}^{\circ}\text{C}$ above or below this most favorable point covers the limits for their most vigorous growth. Few bacteria grow well under 10°C and few over 40°C . 2°C is about the lowest temperature that any bacteria have been found to grow, and 70°C the highest.

In many instances the temperature of the soil in which the bacteria are deposited is the controlling factor in deciding whether growth will or will not take place. Thus nearly all parasitic bacteria require a temperature near that of the body for their development, while many saprophytic bacteria can grow only at much lower temperatures.

Bacteria when exposed to lower temperatures than suffices for their growth, while having their activities decreased, are not otherwise injured; while exposure to higher temperatures than allow of growth destroys the life of the bacteria.

VITAL PHENOMENA OF BACTERIA. The presence of motion is one of the chief evidences of vital activity on the part of bacteria visible in the microscopic field; motility is produced by fine hair-like flagella attached to all motile species; temperature variation, and either an insufficient or excessive supply of oxygen may modify the action.

Bacteria which have the property of emitting light are quite widely distributed in nature, and particularly in media rich in salt, as in sea water, salt fish, etc. The emission of light is a property of the living protoplasm of the bacteria, and is not usually due to the oxidation of any photogenic substance given off by them. Every agent which is injurious to the existence of the bacteria affects this property.

The production of heat by bacteria does not attract attention in our usual cultures because of its slight amount, but careful tests, however, show that heat is produced. The increase of temperature in organic substances when stored in a moist condition, as tobacco, hay, manure, etc., is one, partly at least, due to bacteria.

CHEMICAL EFFECTS. The chemical changes produced by bacteria are chiefly analytic or destructive, the formation of simpler from more complex bodies.

Bacteria are able to construct their body substances out of various kinds of nutrient materials and also to produce fermentation products or poisons, and they are able to do these things either analytically or synthetically with almost equal ease.

This ambidextrous metabolic power exists, according to Huetpe, among bacteria to an extent known as yet among no other living things.

The chemical effects which take place from the action of bacteria are influenced by the presence of oxygen; the access of pure atmospheric oxygen makes the life processes of most bacteria more easy, but is not indispensable when available substances are present which can be broken up with sufficient ease.

In the presence of oxygen the decomposition products which are formed by the attack of the anaërobic bacteria are further

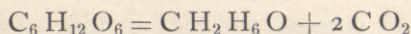
decomposed and oxidized by the aërobes; they are thereby rendered as a rule, inert, and consequently harmless. These facts in connection with anaërobiosis, are of great importance in technical biology and pathology, since under strictly anaërobic conditions, any secondary oxidation of the primary decomposition products is impossible, the latter accumulates without formation of by-products.

Many parasitic bacteria are found to produce far more poisons in the absence of air than in its presence.

Fermentation is a term which is differently used by different observers, but is best defined as a chemical decomposition of an organic compound, induced by living organisms or substances contained within them, or by chemical substances thrown off from the bacteria.

In the first the action is due to the growth of the organism producing the ferment, as in the formation of acetic acid from alcohol by the action of the vinegar plant, and in the second the enzyme causes a structural change without losing its identity, as in digestion.

All fermentation has for its object the acquisition by the organism of a store of energy. This is accomplished in either of the ways mentioned. The simplest and commonest example of decomposing fermentation produced by an enzyme is that of sugar.



Bacteria which develop in the absence of oxygen are especially in need of this source of oxygen. Opposite to this, and far less common, is oxidizing fermentation, as in the production of acetic acid from alcohol. Here the energy is acquired not by the decomposition but by the oxidation of the alcohol.

The proteolytic or peptonizing ferments which are somewhat analogous to pepsin and trypsin, being capable of changing the albuminous bodies into soluble and diffusible substance, are very widely distributed.

The liquefaction of gelatine, which is chemically allied to albumin, is due to the presence of a proteolytic ferment or enzyme.

Fermentation yields products that are poisonous to the ferment; hence fermentation ceases when the nutriment is exhausted or the fermentation is in excess. Different kinds of fermentations obtain specific names, according to the product as acetic, alcoholic, ammoniacal, butyric, lactic, and viscous.

The conversion of urea into carbonate of ammonia affords special evidence of the production of alkaline substances by bacteria; it is a property though which is not very wide spread.

Chromogenesis is a phenomenon which occasionally accompanies fermentation, and manifests itself principally in the formation of red and yellow, blue, and violet pigments.

Putrefaction: By putrefaction is understood in common parlance every kind of decomposition due to bacteria, which results in the production of malodorous substances. Considered in a scientific way, putrefaction depends upon the decomposition of complex organic compounds, albuminous substances and the like, which are frequently first peptonized and then further decomposed.

Typical putrefaction occurs only when oxygen is absent or scanty; the free passage of air through a culture of a putrefactive organism—an event which does not take place in natural putrefaction—very much modifies the process: first, biologically, as the anaërobic bacteria are inhibited, and second by the action of the oxygen on the products or by-products of the aërobic and facultative anaërobic bacteria.

As putrefactive products we have peptone, ammonia, and amines, leucin, tyrosin, and other amide substances, oxy-fatty acids, indol, skatol, phenol, and finally sulphuretted hydrogen, carbonic acid, hydrogen, and possibly marsh gas.

Aromatic products of decomposition also result from bacteria not belonging to the putrefactive group; some twenty-three varieties according to Lehman give the indol reaction, most of the spirilla produce indol and some also produce phenol.

Sulphuretted hydrogen is a common bacteria product, but is formed most frequently from albuminous substances, and hence is a common accompaniment of putrefaction.

A further step in the decomposition of complex organic compounds is that known as nitrification, which is produced by a small, special group of bacteria, cultivated with difficulty, which do not grow on our usual culture media.

From the investigations of Winogradsky it would appear that there are two common micro-organisms present in the soil, one of which converts ammonia into nitrites, and the other converts nitrites into nitrates. This process is of extreme importance to plant life, since it brings the nitrates within reach of higher vegetation which would otherwise be impossible.

A number of bacteria have the power of converting nitrous and nitric acids into free nitrogen, which is of practical importance since by their action large quantities of nitrates in the soil, may become lost as plant food by being converted into nitrogen.

Nitrogen combination: Certain bacteria, among which is the bacillus radicocola isolated by Beyerinck, have the power of assimilating nitrogen from the air. These bacteria are found in the small root-nodules of various leguminous plants (pease, clover, etc.), and can be obtained from these in cultures. By aid of the root bacteria, which gain entrance to the roots and there produce this nodular formation, the leguminous plants are enabled to assimilate nitrogen from the atmosphere thus yielding harvests of grain, etc., which are highly nitrogenous, upon soils which are naturally poor in nitrogen.

Aside from the various bacterial products mentioned, a large number of basic crystalline substances have been recognized, as products of bacterial growth. These are now commonly known as *ptomaines* or putrefactive alkaloids; they have been separated in connection with decomposing fluids—meat, fish, old cheese, and milk undergoing decomposition, and when absorbed by the system are capable of producing very serious disease changes. In addition to the above, bacterial proteines, tox-albumens, or toxins have been separated, especially in connection with the parasitic bacteria,

which cause many of the characteristic symptoms of the infectious diseases.

ARTIFICIAL CULTURE MEDIA. Our knowledge of bacterial activity has been greatly facilitated through the study of individual micro-organisms, by the isolation of separate varieties and cultivation in artificially prepared culture media. In order to determine the number of living bacteria in any substance and their nature, it becomes necessary to cultivate and isolate them. All of the nutrient media used for the growth of bacteria must have, as noted before, food containing the necessary carbon, nitrogen and mineral substances in a form easily assimilated and in the proper concentration. The pathogenic bacteria nearly all require for growth peptone, albumens, and sugar. Special media are required for certain varieties of bacteria. The most common of the nutrient media used in bacteriologic work will be described here.

Nutrient bouillon or broth. One part of finely chopped, fresh, lean meat is macerated in two parts of water and put in an ice chest for from 18 to 24 hours. The infusion is strained when cold, through a fine cheese cloth, and to the filtrate 1 per cent. of peptone and 0.5 per cent. of sodium chloride are added. The medium is then warmed for some minutes until the peptone is dissolved, and then exposed to live steam either without pressure in the Arnold steam sterilizer for thirty minutes, or in the autoclave at one atmosphere of pressure for fifteen minutes, or boiled over a free flame for ten minutes.

While still hot it is filtered through filter paper or through absorbent cotton, and the reaction is tested and sufficient hydrochloric acid or sodium hydrate added to give it the desired reaction, which is for most bacteria slightly alkaline to litmus. If the liquid is clear it is put into flasks and tubes and sterilized; if not clear, the white of one or two eggs is added to the fluid after cooling it down to 55°C. After thoroughly mixing the eggs, the bouillon is boiled briskly for a few minutes and then again filtered and distributed in flasks and tubes and put in the Arnold sterilizer for an hour on each of

two consecutive days, or in the autoclave for twenty minutes, for sterilization.

Nutrient gelatine. To the bouillon already prepared as described add 10 per cent. of sheet gelatine and neutralize. Add the whites of two eggs for each litre and boil for a few minutes. Filter, place in tubes or flasks, and sterilize.

Nutrient agar. This is prepared by adding to stock bouillon 1 to 2 per cent., as desired, of thread agar, melting it by placing over a free flame or in the autoclave or steam sterilizer. When the agar is brought into solution over a free flame there may be considerable loss of fluid by evaporation. This should be compensated by adding additional water before boiling. Agar may be added directly to the meat infusion along with peptone and salt. Indeed, this is an advantage, as agar-agar is very difficult to bring into solution, and is not injured in the least by prolonged boiling.

Glycerine agar is simply nutrient agar plus 3 to 5 per cent. of glycerine.

Nutrient agar begins to thicken at a quite high temperature, and should be filtered as hot as possible.

SEPARATION OF BACTERIA. In examining any material to determine the presence of bacteria, we are apt to find that instead of one variety of bacteria only, there are a number present. If such material is placed in fluid media contained in test tubes, we find that the different varieties all grow together and become hopelessly mixed. When, on the other hand, the bacteria are placed on solid media, such as agar or gelatine, they develop about the spot where they are inoculated. If different varieties, however, are placed too near together, they overgrow one another; a greater surface is therefore advisable than is furnished by the ordinary test tube, and this need is met by pouring the media while warm on flat, cool, glass plates or into shallow so-called petri dishes.

Plate cultures. In making plate cultures two methods are carried out. In the first the material with its contained bacteria is scattered throughout the fluid before it hardens; in the second it is streaked over the surface of the medium after it

has solidified. Nutrient agar and nutrient gelatine, the two substances used for plate cultures, differ in two essential points, which cause some difference in their uses. Nutrient 1 per cent. agar melts at a high temperature and begins to thicken at about 36°C . It is not liquified by bacterial ferments. Nutrient 10 per cent. gelatine melts at the low temperature of about 23°C and solidifies at a point slightly below that. It is liquefied by many bacterial ferments. Great care is necessary in inoculating fluid nutrient agar for plate cultures so that in cooling it to a point which will not injure the bacteria, about 41°C , it is not allowed to cool too much and thus solidify and prevent the pouring it into plates. The material to be examined, be it milk, water, soil, or sewage, is added to the liquefied media in whatever quantity is thought to be proper.

After inoculation the contents of the tubes are thoroughly shaken and poured quickly into round, flat bottomed glass dishes (sterilized), the covers of which are removed for the required time only. The bacteria are now scattered throughout the fluid, and as it quickly solidifies they are fixed wherever they happen to be, and thus as each individual multiplies clusters are formed about it at the spot where it was fixed at the moment of solidification. The number of colonies of bacteria thus indicates to us roughly the number of living bacteria in the quantity of fluid added to the liquid agar. Nutrient gelatine is used exactly as agar, except that, as it does not congeal until cooled below 22°C , there is no fear of its cooling too rapidly. In order not only to count the number of colonies which develop, but also to obtain a characteristic growth, it is desirable not to have them too near together. As it is impossible to determine accurately the number in any suspected fluid, it is usual to make a set of three or four different plates, to each of which, a different amount of material is added, so that some of the four will have the required number of colonies. Measured quantities of the diluted material can be transferred most accurately through a sterilized long glass pipette graduated to hun-

dredths of a cubic centimetre, or, more roughly, by a platinum loop of known size. The colonies growing in the third and fourth plates will be separated so as to be individually studied and counted without difficulty.

Counting of Colonies. For this purpose the dishes are covered by a glass plate, ruled in larger and smaller squares. With a hand lens the colonies in a certain number of squares, are counted and then the number for the whole contents estimated. The counting of the colonies represents a quantitative analysis of a suspected material, since the number of the colonies approximately determines the number of bacteria present.

Each colony forms a pure culture of a separate species of bacteria, and transplantations can be made from these primary growths to tubes of the different media, perchance also inoculated into animals, and there its different characteristics may be ascertained. This qualitative analysis is attended by a great deal of labor and expenditure of time; very frequently, as in the examination of water, certain kinds of bacteria as the typhoid bacillus or the spirillum of Asiatic cholera, are sought for, which greatly simplifies the examination.

The introduction of solid culture media and the plate culture method, has made it possible to separate the many different kinds of bacteria that are now known to us, and placed the study of bacteriology on a more accurate and scientific basis.

The interest of the engineer is mainly centered in the occurrence of bacteria in nature, and the different ways by which the natural media may become contaminated.

BACTERIA IN WATER. The bacteriology of water has now assumed considerable importance, and its examination by bacteriologic methods has become a routine part in analysis for hygienic purposes. The bacterial flora of natural waters is a very varied one. In surface waters, such as streams, ponds, and shallow wells, the organisms met with are largely derived from the air and soil through which the water has

passed. When uncontaminated from human or animal sources, by the air of towns, sewage, manure, etc., they consist of bacilli, the majority of which are chromogenic, non-liquefying, and develop on culture media at a temperature of 22°C , or thereabouts only, not at blood-heat; also of some sarcina and a few micrococci. When, however, the water passes through cultivated lands, or receives sewage, the number of organisms is enormously increased; a large proportion of them liquefy gelatine and develop at blood-heat, while members of the colon group appear more or less numerous. Whereas water from shallow wells has a bacterial content nearly as great as the surrounding surface water, that from deep wells is remarkably free from organisms. The following table illustrates the number of organisms which may be met with in water from different sources:

<i>Source.</i>	<i>Number of organisms per cubic centimetre.</i>
Freshly fallen snow	34—38
Rain water	4—5
Rhone, above Lyons	75
Rhine, at Mühlheim	20,000
Thames, at Hampton (Frankland)	2000—90,000
Deep well in the chalk (Frankland)	7—21
Surface well	1200
Lake of Luzerne	8—50
Iowa River at Iowa City	120
Filtered water supplied to London (Frankland)	
	Average not more than 100
Sewage (Frankland)	26,000,000.

The number of bacteria in water varies considerably with its source, at different seasons, and under different climatic conditions.

The bacteriologic analysis of water may afford valuable indications as to the purity or otherwise of a water; it cannot, however, supplant in any way the chemic analysis; the two methods supplement each other and should be worked side by side. The search for pathogenic organisms, though most important, is not everything, for not only are they extremely difficult to find, and a negative result is therefore often of

little value, but the source of infection may have ceased and the pathogenic organisms have disappeared before the water is submitted for examination.

The number and character of the organisms must also be taken into account, though at the same time caution is required in interpreting the results, for it should be clearly borne in mind that waters differ bacteriologically as well as chemically, and that to reject a water because it contained rather more than the recognized number of organisms would be equivalent to condemning it, say, for an excess of chlorine, the strata and geological features of the district being unknown. Strictly speaking, the mean bacterial content of a water or supply should be ascertained, and then any departure from this mean or average may yield valuable information. The full value of a bacteriological examination, for example, would be seen if systematic analyses under similar conditions were made at the supply from the filter-beds or at the supply as it leaves the water company's premises. In a year or so a standard of the average number of bacteria normally present in the water at different seasons would be arrived at, and by continuing the examinations at regular intervals, any sudden and marked departure from the standard so ascertained would indicate that something was wrong with the filter-beds or elsewhere, and would indicate the necessity for an investigation.

Bacteriologic Examination of Water. The specimen of water should be collected in clean bottles, sterilized preferably by heat, and it should of course be a representative specimen. The routine bacteriologic examination of the specimen falls under the following sections:

1. The number of organisms present in a given volume.
2. The ratio of organisms liquefying gelatine to those which do not.
3. The number of organisms present in a given volume which will develop at blood-heat.
4. The search for the colon bacillus, typhoid bacillus, or other pathogenic species.
5. The virulence of a peptone-water culture.

1. The number of organisms present in a given volume, 1 c.c. being that usually adopted, is estimated by means of gelatine cultivations. The gelatine tubes are melted in the ordinary way and inoculated with a measured volume of water. The colonies which develop in each plate are counted, and their number represent, roughly, the number of organisms present in the specimen. The results are reduced to the number of organisms per cubic centimeter. As already indicated, no definite conclusions can be drawn from the number of organisms present unless the source of the water and the conditions under which the sample was taken are accurately known. Koch laid down as a standard that a good water should not contain more than 100 organisms per c.c., but under ordinary conditions water rarely comes up to this standard, and anything less than 500 organisms per c.c. may be regarded as fairly good.

2. The ratio of organisms liquefying gelatine is readily ascertained from the gelatine plates employed to enumerate the organisms. It is stated that the ratio of liquefying to non-liquefying forms should not exceed one to ten. The normal bacteria of water are largely non-liquefying, but if the water be contaminated with sewage the number of liquefying organisms becomes largely increased.

3. The number of organisms which will develop at blood-heat, is of considerable import, since the majority of the normal bacteria of water do not develop at blood-heat, whereas those derived from sewage to a large extent do. Hence, if by the gelatine plate method a large number of organisms are found to develop at 22°C, and a large proportion of them do not develop at blood-heat, the water would be regarded as of much better quality than if this were not the case. In order to estimate the number of organisms which develop at blood-heat, agar plates are prepared with about 0.5 c.c. of the water, and incubated at 37°C for 24 hours, and the colonies are then counted.

4. The search for pathogenic organisms, especially the typhoid bacillus, is a difficult matter, and is not undertaken

unless specially required. The colon bacillus should always be sought for, as its presence is a fair criterion of contamination with sewage, surface drainage, or animal excrement. For its detection the so-called fermentation tubes are useful; these consist of agar tubes containing 2 per cent. of glucose, and since the colon bacillus is a fermentative organism, its growth in glucose agar is accompanied by the formation of carbonic acid gas, appearing as bubbles in the depths of the media. The typhoid bacillus does not produce fermentation. As the colon bacillus is such a ubiquitous organism some care must be exercised in condensing a water because of its presence; even the purest waters may contain it in small numbers. If the colon bacillus is met with in large quantities it is, to say the least, very suspicious; if any considerable number of colonies are met with (in the gelatine plates used for enumeration) the water should be condemned.

It is needless to say, that the presence of the typhoid bacillus or comma bacillus of cholera is sufficient to condemn a water.

5. *The virulence of a peptone-water culture.* If sufficient peptone and salt be added to a measured volume of the water to form a one per cent. solution of the former, and a one-half per cent. solution of the latter, the mixture incubated at 37°C for 24 hours and injected intraperitoneally into a guinea pig, a good water is stated not to kill, whereas a bad one does. The amount to be injected is 2 c.c., and death should ensue within 48 hours.

BACTERIOLOGY OF THE AIR. Just as in water, the bacteria in the air vary considerably at different times and seasons, under different conditions, and in various localities. The species met with are mostly saprophytes, consisting largely of chromogenic forms.

It is not easy for micro-organisms to become diffused through the atmosphere; they are incapable of a voluntary rising, and cannot be torn from a fluid or moist solid medium by a strong current of air. The medium on which they are growing must dry up completely and crumble into fine dust.

before they can be distributed through the agency of air currents. Micro-organisms are more numerous in the air during summer and autumn. After heavy rains the air is much freer from organisms. On high mountains, organisms are nearly absent from the air. Microbes are much fewer in the air of the country than in that of towns. In the words of Cowper, "God made the country, and man made the town."

SOIL. The upper layers of the soil contain large numbers of organisms, chiefly bacilli. The species are very varied; among pathogenic ones may be named the bacilli of tetanus (lockjaw) and of malignant œdema (black leg). The nitrifying organisms are very abundant in the soil. Below five or six feet, aërobic organisms are scanty, but the anaërobic and thermophilic ones are still met with. The number of organisms present in the soil is variable, from 200,000 to 4,500,000 in ordinary earth, while in dirty and busy streets there may be as many as 1,000,000,000 per gram.

SEWAGE. Sewage is exceptionally rich in organisms, but the numbers present are variable. Jordan, in Massachusetts, found an average of 708,000 per c.c. Laws and Andrews found from 905,000 to 11,216,000, the latter being the highest number obtained. The number of organisms naturally varies at different seasons, and with the amount of dilution. The organisms are very varied. A few micrococci are met with, but bacilli, especially *liquefying forms*, largely predominate. Many anaërobic sporing bacilli are also found. The colon bacillus numbers from 20,000 to 200,000 per c.c., and the other varieties of bacilli number 200,000 to 2,500,000 per c.c. Bacteria introduced into sewage are probably soon suppressed by the predominant species in the sewage. The air of well ventilated sewers differs but little from that of the external air, and the organisms in it contrast with those of sewage by the abundance of moulds.

PURIFICATION OF WATER AND SEWAGE. Air and soil do not play the role in the transmission of disease formerly ascribed to them, but they frequently serve as media for the conveyance of disease germs. Of much greater importance

is water, concerning whose essential participation in the causation of epidemics, frequent and ample evidence is noted. A perfectly pure water signifies one free from bacteria and such substances as are suitable for bacterial growth. The furnishing of a healthfully pure water supply is one of the most important problems within the province of the sanitary engineer.

Self purification of water. Pettenkoffer has taught us, that if renewed contamination does not take place, streams and water can purify themselves. The micro-organisms settle and are carried to the bottom, partly with the constituents suspended in the water and with the insoluble earthy combinations that form from calcium and magnesium bi-carbonates after escape of the carbon dioxide. Light also in a high degree exerts an injurious influence upon the micro-organisms present in water down to a depth of two meters. The organic substances contained in water are gradually consumed by bacteria and algæ.

That river water which has been fouled by sewage will, in the course of a few miles, through the dilution of additional supplies, through sedimentation and through oxidation, become greatly purified is an indisputable fact. The increase in bacteria which occurs from contamination is also largely or entirely lost after 10 or 20 miles of river flow. That it is rather a doubtful security to depend on river purification is proved by the following: In the city of Lowell, Massachusetts, an alarming epidemic followed the pollution of the Merrimac river three miles above by typhoid feces, and six weeks later an alarming epidemic attacked Lawrence, nine miles below Lowell. It is estimated that the water took ten days to pass from Lowell to Lawrence and through the reservoirs. As typhoid bacilli may live for twenty-five days in water, the Lawrence epidemic is easily explained.

Purification of water on a large scale. Surface waters, if collected and held in sufficiently large lakes or reservoirs, usually become so clarified by sedimentation as to require no further treatment so far as its appearance goes. The collec-

tion of water in large reservoirs allows time for the pathogenic germs to perish through light, and antagonistic bacteria and other deleterious influences. The filtration of water effects a very marked purification, taking out often 99 per cent. in those best constructed, and 90 per cent. and over in those commonly used in cities.

The construction of filters can not be minutely entered upon here; they consist, as a rule, of several layers, beginning with fine sand and then smaller and larger gravel, and finally rough stones; they may be arranged in the form of sand beds or basins; or in large cylinders or steel shells as seen in the Davenport, Iowa, filter plant; with a new filter a certain time elapses before the best results are obtained; this seems to wait for the formation of a film of organic material or *bacterial jelly* on the sand, which is full of nitrifying bacteria. The two essential factors therefore which control this form of filtering process are a mechanic and a biologic one; the passing of water through a porous substance like sand, gradually frees it of deleterious substances, and among them the bacteria, but the removal of bacteria would be quite imperfect were it not for the accumulation of organic material on the surface, and the arrest of all those micro-organisms which exist best where light, oxygen, and organic matter is present, thus forming on the surface of the filter a film which is highly essential, and in fact the most important element, for good filtration.

Domestic purification. Water which requires private filtering should not be supplied now for drinking purposes. Unhappily, however, it often is. Filters may be divided, roughly, into those for high and low pressure. The former are directly connected with the water main, while the others simply have the slight pressure of the column of water standing in the filter.

Many high pressure filters contain animal charcoal, silicated carbon, etc., either in a pressed condition or in one porous mass.

These filters remove much of the deleterious matter from

the suspected waters, but the majority cannot be depended upon to remove all bacteria. Even those which are equipped for self-cleansing become in a little while foul, and, if not cleaned and sterilized, unfit for use. The best of this class are the Berkefeld and Pasteur filters. These yield a water, if too great pressure is not used, almost absolutely free from bacteria, and if they are frequently and *properly* cleaned by boiling or baking, they are reliable. A large Berkefeld filter will allow sixty gallons of water to pass per hour. The Pasteur filter is more compact and slower. From the best Pasteur filters sterile water may be passed for two to three weeks without resterilizing; from the Berkefeld only a few days. Animal charcoal is not a good substance for permanent filters, as bacteria grow well in it.

Whenever water is suspected, and there is any doubt as to the efficiency of the filters, or when a filter is not at hand, it should be boiled for ten minutes; this will destroy all bacteria.

For this form of domestic purification, the ingenious sterilizing apparatus of Mr. John Tarbes, described by Prof. A. V. Sims, of the University of Iowa, in "Pure Water, its Value and Attainment," is deserving of special mention. It automatically boils the water with an arrangement for rapid cooling, so that there may, if desired, be less than a one degree difference between the temperature of the water as it flows in and out of the sterilizer. They have capacities of 125 gallons per day and upwards. Practical tests made in the bacteriological laboratory have proven its efficacy in destroying the typhoid bacillus, colon bacillus, and other organisms usually met with in drinking water. After rigorous tests the United States government has adopted them for use in the field with our armies and already purchased over \$75,000 worth. Several European governments are considering their adoption.

DISPOSAL AND PURIFICATION OF SEWAGE. The contamination of brook and river water by sewage is by far the most dangerous form and source of pollution; hence, to dispose of sewage, render it harmless, and purify the contaminated water is a vital problem of the time. Several processes for

the purification of sewage are in vogue, all of which have their benefits, and also their necessary limitations. Great advances have been made in our knowledge of the changes which sewage undergoes in purification, by means of numerous recent bacteriologic and chemical investigations, from which not a few conclusions of wide-reaching importance have been established.

Of the methods now in use, should be mentioned:

1. Sewage farms, or the land treatment of sewage, known also as broad irrigation.
2. Precipitation process.
3. Filtration and nitrification.
4. Bacteriolysis.

1. The land treatment of sewage is perhaps the simplest method of purifying sewage; it is a means of land irrigation with a certain degree of intermittent filtration. A suitable soil is necessary for the process; clay soils are specially unsuitable. Nitrification being the essential influence in the disintegration of the organic material on the surface, and nitrifying organisms are not only scarce in clay soil, but the latter lacks porosity, this preventing frequent aëration and supplies of oxygen. The soil in this instance acts as a filter, and the mechanical with the biologic feature prevails here as in the sand gravel filters. The irrigation of the land has made fertile tracts out of barren wastes. The farm at Gennevilliers, receiving the sewage of Paris, offers a striking example of this fact. The largest sewage farm in the world is that at Berlin. In March, 1895, the available area for irrigation was 22,881 acres, with a population draining to it of about 1,750,000 inhabitants. Water collected in drains, placed eight or nine feet beneath the surface, is a filtered water and contains but comparatively few organisms and is suitable for drinking purposes. The distance of the drains below the surface depends largely on the character of the material, and may usually be much less than that given above.

2. *Precipitation.* This process signifies the deposition of the insoluble matter in suspension in sewage, together with a

certain proportion of the organic matter in solution, by the addition of some chemical, like alum, copperas, or lime, which forms insoluble compounds with it, and at the same time deodorizes it. It is generally admitted that by precipitation practically only the solid matter in suspension is removed, and the removal of bacteria is not sufficient, to render the effluent safe.

3. Filtration of sewage is much on the same plan as sewage farming, except that it implies the use of filters or basins, specially prepared for the purpose. It represents in reality a biological sewage filter, being based on the principle of nitrification. The biological element greatly predominates over the mechanical, and the rate at which the nitrification occurs controls the rapidity of the purification, so that a certain amount of sewage can only be disposed of on a certain area.

4. *Bacteriolysis*. The powerful liquefying and solvent actions of the bacteria in sewage have suggested a means of dealing with sewage so as to make use of these properties and render sewage disposal an automatic process, and to which the term of bacteriolysis has been applied. Such are the Scott-Moncrieff and septic tank processes. In the former the sewage is led by channels through beds of flint and coke, by which a large surface is presented to the organisms; in the latter the sewage slowly passes through a tank and gradually becomes dissolved, liquefied and to a large extent purified. The rapidity with which the various bacteria liquefy the solid organic matter varies considerably, and it is claimed that those bacteria which live in the absence of air are the most active liquefying organisms.

Mr. Sims Woodhead found that in 1 c.c. of Exeter, (Eng.) crude sewage there were over 1,000,000 organisms which were anaërobic, and 5,500,000 which were aërobic. Of the 1,000,000 anaërobic 300,000 were found to be liquefying organisms, and of the 5,500,000 of aërobic, 500,000 were also found to be liquefying, so that the proportion of liquefying organisms was found to be greater among anaërobic than aërobic bacteria.

In a recent discussion before the British Medical Association, Dr. A. C. Houston offered some valuable contributions as to whether pathogenic bacteria were destroyed by the sewage treatment. By selecting specific organisms like the colon bacillus, cholera spirillum and some pus-producing organisms, he was able to follow them entirely through the bacteriolytic process, and he considers that the death of these microbes is certainly a question of days and probably of some weeks, when exposed to these influences. It is evident from these experiments, that time is an all-important factor, and that it is doubtful if any bacterial process in practical operation at the present time treats or detains the sewage for a sufficiently long period to allow of the complete destruction of all pathogenic germs by bacterial agencies. But the filtering of the sewage by biological, nitrifying filters, after passing through the septic tank process, helps, and generally eliminates all resisting pathogenic organisms.

The process of filtration and destroying of organisms on a large scale is still open to much improvement, and worthy of the best efforts of the sanitary engineer.